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Identification and Manipulation of Soil Properties To Improve the Biological Control Performance of Phenazine-Producing *Pseudomonas fluorescens*

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***Pseudomonas fluorescens* 2-79RN$_{10}$ protects wheat against take-all disease caused by *Gaeumannomyces graminis* var. *tritici*; however, the level of protection in the field varies from site to site. Identification of soil factors that exert the greatest influence on disease suppression is essential to improving biocontrol. In order to assess the relative importance of 28 soil properties on take-all suppression, seeds were treated with strain 2-79RN$_{10}$ (which produces phenazine-1-carboxylate [PCA$^+$]) or a series of mutants with PCA$^+$ and PCA$^-$ phenotypes. Bacterized seeds were planted in 10 soils, representative of the wheat-growing region in the Pacific Northwest. Sixteen soil properties were correlated with disease suppression. Biocontrol activity of PCA$^+$ strains was positively correlated with ammonium-nitrogen, percent sand, soil pH, sodium (extractable and soluble), sulfate-sulfur, and zinc. In contrast, biocontrol was negatively correlated with cation-exchange capacity (CEC), exchangeable acidity, iron, manganese, percent clay, percent organic matter (OM), percent silt, total carbon, and total nitrogen. Principal component factor analysis of the 16 soil properties identified a three-component solution that accounted for 87 percent of the variance in disease rating (biocontrol). A model was identified with step-wise regression analysis (R^2 = 0.96; C*p* statistic = 6.17) that included six key soil properties: ammonium-nitrogen, CEC, iron, percent silt, soil pH, and zinc. As predicted by our regression model, the biocontrol activity of 2-79RN$_{10}$ was improved by amending a soil low in Zn with 50 μg of zinc-EDTA/g of soil. We then investigated the negative correlation of OM with disease suppression and found that addition of OM (as wheat straw) at rates typical of high-OM soils significantly reduced biocontrol activity of 2-79RN$_{10}$.**

Root-associated bacteria (e.g., *Pseudomonas* and *Bacillus* spp.) have been shown to protect crops against a broad spectrum of soilborne fungal pathogens (13). However, commercial application of these bacteria for biocontrol has been hampered in large part because of their inconsistent performance between field locations (45). Such variability has been attributed to different causes, including host plant genotype (37, 44), agricultural practices (28), mutation of the biocontrol strain (7), pathogen resistance to biocontrol mechanisms (23), and vulnerability of the biocontrol strain to pathogen defense mechanisms (8). For biocontrol strains of *Pseudomonas fluorescens*, antibiotic production often plays a central role in disease control (40). The effects of various minerals, growth factors, carbon and nitrogen source, pH, and temperature on antibiotic production of biocontrol strains of *P. fluorescens* in defined liquid media have been examined (9, 29, 30). The effect of soil pH on the biocontrol efficacy of *P. fluorescens* also has been described (26). However, analysis of the overall abiotic soil environment and its impact on biocontrol is lacking.

The first objective of this study was to identify edaphic parameters that influenced, positively or negatively, the biocontrol activity of *P. fluorescens*. As a model, we selected biocontrol of take-all disease of wheat using *P. fluorescens* strain 2-79. Take-all, caused by the ascomycetous fungus *Gaeumannomyces graminis* var. *tritici*, is an important root and crown rot disease of wheat worldwide, particularly in relatively moist regions or under irrigation (5). Strain 2-79 was isolated from wheat roots grown in a take-all decline soil, one that develops natural disease suppressiveness after long-term wheat monoculture. Intensive study since the early 1980s has demonstrated that 2-79 aggressively colonizes wheat roots (43) and suppresses take-all disease in the field (46). However, as is typical for many biocontrol agents, the effectiveness of 2-79 in the field varies from location to location (45). Our second objective was to compare the effect of soil properties on the biocontrol activity of strains derived from 2-79, which differed in their ability to produce the antibiotic phenazine-1-carboxylate (PCA). In previous studies with 2-79, genetic analysis indicated that production of PCA in the rhizosphere is a primary mechanism of biocontrol accounting for up to 90% of disease suppression (38, 39, 40, 41), with other antifungal factors, including a fluorescent siderophore and anthranilic acid, making minor contributions to biocontrol activity (12). However, in other biocontrol systems, such as suppression of *Fusarium* wilts with fluorescent pseudomonads, siderophore-mediated iron competition in low-iron environments is an effective mechanism of disease control (22). Although a PCA-deficient, anthranilate-producing mutant (FM13) of the biocontrol strain

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TABLE 1. *P. fluorescens* 2-79 and derivatives

Strain	Relevant characteristics[a]	Reference
2-79	Phz^+ Flu^+ Aff^+ Rif^S	46
2-79RN_{10}	Phz^+ Flu^+ Aff^+ Rif^r Nal^r (spontaneous)	46
2-79RNL3	2-79RN_{10} with a *lacZ* fusion generated with Tn*3*Ho-Ho1	6
2-79-59.34	2-79RN_{10}::Tn*5* Phz^+ Flu^- Aff^+	12
2-79-892B	2-79RN_{10} Phz^- (NTG) Flu^+ Aff^+	47
2-79-B46[b]	2-79RN_{10}::Tn*5* Phz^- Flu^+ Aff	38
2-79-892.224	2-79-892B::Tn*5* Phz^- Flu^- Aff^+	12
2-79-59.34.24	2-79-59.34 Phz^- Flu^- Aff^- (deletion of a 0.6-kb *Eco*RI genomic fragment carrying sequences that are essential for phenazine biosynthesis)	12

[a] Phz^+ Flu^+ Aff^+, produces PCA, fluorescent siderophore, and anthranilic acid, respectively; Rif^S, sensitive to rifampin; Rif^r Nal^r, resistant to rifampin and nalidixic acid, respectively, at 100 μg/ml; Phz^- Flu^- Aff^-, deficient in production of phenazine, fluorescent siderophore, and anthranilic acid, respectively; NTG, *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine.

[b] The loss of PCA production in *P. fluorescens* 2-79-B46 results from a transposon insertion into the regulatory gene *gacS* (L.S. Thomashow, unpublished data).

P. aeruginosa PNA1 was not as effective as the parental strain in suppression of Fusarium wilt of chickpea, it was as effective as the wild-type strain in biocontrol of *Pythium* damping-off of bean (1). Our rationale for including several derivatives of 2-79 was to determine if the reduced effectiveness against take-all of mutants that could not produce PCA that was observed in previous studies would occur over a range of different soils. Our third objective was to determine if our insight regarding soil properties had practical applications for improving biocontrol. To do this we amended soil with zinc or organic matter, soil factors that were identified in our study as favorable and unfavorable for disease suppression, respectively, by strain 2-79.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains and relevant phenotypes are described in Table 1. *P. fluorescens* strain 2-79RN_{10} (43) is a spontaneous rifampin-resistant, nalidixic acid-resistant derivative of 2-79 (NRRL B-15132), which was originally isolated from roots of wheat grown in a take-all decline soil from Lind, Wash. (46). Derivatives of strain 2-79RN_{10}, deficient in production of PCA, fluorescent siderophore, and/or anthranilic acid and previously generated by transposon, deletion, and nitrosoguanidine mutagenesis (12, 38, 47), were used also. The loss of PCA production in *P. fluorescens* 2-79-B46 results from a transposon insertion into the regulatory gene *gacS* (L. S. Thomashow, unpublished data).

Seed bacterization. Bacteria were applied to surface-disinfested spring wheat seeds (*Triticum aestivum* L. 'Fielder') according to the method of Ownley et al. (26). Briefly, bacteria were cultured on nutrient broth yeast extract agar (42) and incubated at 27°C for 48 h. The bacterial lawns were suspended in 5 ml of sterile deionized water, and then 2 ml of the suspension was inoculated onto each of two King medium B (20) agar plates and incubated at 27°C for 24 h. Bacteria from two King medium B plates were suspended in 20 ml of medium-viscosity 0.5% (wt/vol) methylcellulose (MC) (Sigma, St. Louis, Mo.). Seeds were coated with bacteria by mixing 2.5 ml of bacterial suspension with 5 g of seed. The coated seeds were dried for 1.5 to 2 h under a stream of sterile air at room temperature. This process consistently yielded densities that ranged from 10^7 to 10^8 CFU/seed, which were verified by dilution plating before planting. The control was treated with 0.5% MC, and no bacteria were detected by dilution plating.

Pathogen inoculum. *G. graminis* var. *tritici* (isolates R3-111a-1 and SCS) was prepared as described previously (10, 26) on autoclave-sterilized whole oat kernels (250 g of oats and 250 ml of deionized water per liter flask, 4 weeks in light at 21 to 25°C). Colonized oat grains were air dried, ground in a blender, and sieved. Particles of 0.25 to 0.5 mm were collected for use as inoculum. A mixture of the two virulent isolates of *G. graminis* var. *tritici* was used to minimize possible effects from differential sensitivity of the pathogen to the biocontrol agent (23).

Soils. Ten soils representative of those in the wheat-growing region of the Pacific Northwest were included in this study. Quincy fine loamy sand and Larkin, Palouse, Puget, Ritzville, Thatuna, Walla Walla, and Woodburn silt loams were collected from sites in the state of Washington state near Pasco, Rockford, Pullman, Mt. Vernon, Lind, and Pullman and sites in Oregon near Pendleton and Corvallis, respectively. Shano silt loams were collected from two distinct sites in Washington state, Quincy and Moses Lake. For each soil, 28 chemical and physical characteristics were determined by the University of Idaho Soil Testing Laboratory, Moscow (Table 2). Soils were collected from the upper 30 cm of the profile, sieved (2.0-mm-pore-size mesh), steam treated (1 h at 95°C), and air dried on a bench top at room temperature before use. The take-all pathogen is sensitive to antagonism by a wide range of soil microorganisms (48); thus, soils were steamed to "normalize" the impact of biotic factors on take-all disease among the 10 soils tested.

Take-all suppression assay. Tube-assays were conducted in a growth chamber as previously described (10, 26). Pathogen inoculum was mixed into soil at 0.45% (wt/wt) based on soil dry weight. Plastic tubes (2.5-cm diameter by 16.5-cm height; Stuewe and Sons, Corvallis, Ore.), held upright in racks (200 per rack), were filled with a cotton plug, 25-cm^3 layer of sterile vermiculite, followed by a 15-cm^3 layer of soil infested with *G. graminis* var. *tritici*. Each tube received 10 ml of water with metalaxyl (0.075 g of wettable powder per liter of tap water; Novartis Limited, Greensboro, N.C.) to suppress indigenous *Pythium* spp. Two wheat seeds, treated with either MC or bacteria, were placed on the soil surface in each tube and covered with a 5-cm^3 layer of uninfested soil and a 5-cm^3 layer of sterile vermiculite. The planted cones were incubated in growth chambers at 15°C with a 12-h photoperiod, conditions that are ideal for the development of take-all disease. After emergence, plants were watered twice weekly with 5 ml of 1/3-strength Hoagland's solution (macroelements only, no iron) (14). After 3 weeks, roots were washed free of soil, and take-all severity was rated on a scale of 0 to 8 where 0 = no visible symptoms, healthy plant; 1 = <10% of roots black; 2 = 10 to 25% of roots black; 3 = 25 to 50% of roots black; 4 = 50 to 100% of roots black; 5 = all roots with lesions and lesions at base of stem; 6 = lesions extending up the stem; 7 = plant chlorotic and severely stunted; and 8 = dead plant or nearly so (26).

The take-all suppression assay was arranged as a 10 × 8 factorial in a split-plot design with 10 soil treatments (main plot) and eight seed treatments (MC-treated control, 2-79RN_{10}, and six mutant derivatives) (subplot). Each treatment was replicated five times with 20 seedlings per replicate. The main effects of soil and seed treatment and the interaction were analyzed for significance with the general linear models procedure of SAS (Statistical Analysis Systems Institute, Cary, N.C.). Significant effects were further analyzed with Fisher's-protected least-significant-difference test at $P = 0.05$.

Identification of soil properties involved in biocontrol of take-all. Correlations between the 28 soil parameters measured and the disease suppressiveness of *P. fluorescens* 2-79 derivatives were determined using the SAS correlation procedure. Previously three metabolites (PCA, fluorescent siderophore, and anthranilic acid) were shown to have a role in the biocontrol activity of 2-79 (12). For this reason, data for derivatives with common phenotypes (i.e., all PCA^+, all PCA^- and siderophore$^+$, and all PCA^- and siderophore$^-$ were grouped for the analysis. Due to the primary role of PCA in biocontrol of take-all by 2-79 (12, 38), the relationship between the group of PCA^+ derivatives and key soil factors correlated with their biocontrol activity was further analyzed using principal component factor (PCF) analysis with varimax rotation procedure of SAS. This analysis is an investigative tool useful for identifying patterns of interrelated variables with the aim of selecting a reduced number of variables for further study (19). Soil properties interrelated to take-all disease rating were selected with a preliminary PCF analysis. The selected properties were included in a final analysis to develop the principal component solution that best explained the covariance among take-all disease rating and the soil properties. Loading values greater than or equal to the absolute value of 0.35 indicated significant interrelationships among variables within a principal component. The number of soil properties influencing biocontrol by PCA^+ strains was further narrowed using the step-wise regression analysis procedure of SAS to identify a model that included the least number of soil properties and that best described variation in disease rating (C_p statistic closest to the number of variables included in the model) (25).

Effect of soil amendments with zinc and organic matter on take-all severity and biocontrol. The effects of zinc and organic matter amendments were evaluated in separate experiments. To determine the impact of zinc on biocontrol by 2-79RN_{10}, Woodburn silt loam, the soil with the lowest natural level of zinc (Table 2), was sieved (2-mm-pore-size mesh), steam treated (1 h at 95°C), and air

TABLE 2. Chemical and physical properties of soils

Property	Value for soil[a]:									
	RSL	PSL	LSL	TSL	WSL	WaSL	PuSL	SSL1	SSL2	QFLS
Major element (μg/g)										
Boron	0.27	0.47	0.51	0.52	0.21	0.35	0.54	0.18	0.53	0.37
Nitrogen-ammonium	0.81	3.18	20.3	2.38	2.93	10.7	11.2	4.86	5.60	13.7
Nitrogen-nitrate	2.8	3.3	2.0	36.7	22.0	3.6	40.1	87.5	17.5	17.4
Phosphorus (available)	8.2	13.1	9.7	20.9	7.4	8.0	5.7	10.9	10.6	16.4
Potassium (available)	328	260	384	288	150	528	314	264	324	256
Sulfur-sulfate	5	3	6	4	2	3	5	11	7	16
Extractable cations (mM^+/liter)										
Calcium	5.98	7.20	9.65	9.02	4.95	7.01	6.70	8.62	4.53	6.84
Magnesium	0.88	1.28	1.80	2.39	1.38	2.76	0.88	2.90	1.78	1.61
Potassium	0.939	1.111	1.634	1.069	0.605	2.063	0.952	1.028	1.165	0.723
Sodium	0.140	0.056	0.084	0.120	0.050	0.053	0.067	0.134	0.115	0.180
Soluble cations (mM^+/liter)										
Calcium	0.101	0.061	0.115	0.211	0.159	0.050	0.202	0.559	0.201	0.140
Magnesium	0.021	0.019	0.037	0.086	0.060	0.026	0.043	0.253	0.103	0.060
Potassium	0.023	0.022	0.068	0.052	0.024	0.057	0.075	0.043	0.072	0.037
Sodium	0.044	0.021	0.035	0.056	0.033	0.021	0.037	0.067	0.061	0.086
DTPA extractable (μg/g)[b]										
Copper	1.25	1.67	1.11	1.53	0.63	1.26	4.41	1.95	1.89	0.41
Iron	5.15	78.81	69.95	52.52	78.45	51.31	71.90	54.87	54.85	12.45
Manganese	14.57	107.30	112.20	105.0	80.72	114.70	26.47	77.21	106.20	13.69
Zinc	4.06	3.71	5.79	2.56	0.91	1.64	6.23	4.87	9.23	7.54
pH	8.29	6.05	5.93	6.15	5.17	6.02	6.14	6.27	5.63	8.53
Nitrogen (% total)	0.10	0.13	0.25	0.17	0.18	0.13	0.17	0.10	0.10	0.09
Carbon (% total)	0.57	1.51	2.84	1.63	1.88	1.43	1.89	0.79	0.68	0.55
Organic matter (%)	0.93	2.43	4.42	2.54	2.95	2.30	2.76	1.13	1.14	0.85
CEC (cM^+/kg)[c]	10.2	18.1	20.9	17.0	17.1	16.3	11.4	14.9	10.3	6.4
Exchangeable acidity (meq H^+/100 g)	0.578	7.31	8.67	6.16	7.31	6.35	6.73	3.85	4.42	0.193
Electron conductivity (mho Siemens)	0.30	0.22	0.41	0.79	0.63	0.33	0.60	3.06	1.01	0.98
Particle size distribution										
Clay (%)	6.4	22.4	19.4	18.4	28.4	15.4	22.4	16.4	13.4	7.4
Sand (%)	29.6	13.6	21.6	17.6	13.6	23.6	17.6	53.6	34.6	87.8
Silt (%)	64.0	64.0	59.0	64.0	58.0	61.0	60.0	30.0	52.0	4.8

[a] Soils were classified as the following: Ritzville silt loam (RSL), Palouse silt loam (PSL), Larkin silt loam (LSL), Thatuna silt loam (TSL), Woodburn silt loam (WSL), Walla Walla silt loam (WaSL), Puget silt loam (PuSL), Shano silt loam (SSL1, Moses Lake, Wash, SSL2, Quincy, Wash. and Quincy fine loamy sand (QFLS).
[b] Diethylene triamine pentaacetic acid.
[c] Cation exchange capacity.

dried on a bench top at room temperature prior to addition of zinc amendments. Zinc was added as Zn-EDTA at 15 and 50 μg of zinc-EDTA/g of soil, based on soil dry weight. Control soil received no zinc amendment. Prior to filling plastic tubes, zinc-EDTA treatments were mixed with water and added to soil for a final soil moisture content of 20%. Water was added also to the control soil for a final soil moisture content of 20%. Seeds treated with 2-79RN$_{10}$, 2-79-59.34, or MC were planted into pathogen-infested soil and evaluated for severity of take-all disease in tube assays as described above.

To determine the effect of organic matter on biocontrol by 2-79RN$_{10}$, dried nontreated wheat straw collected from the field was ground in a blender, sieved (2-mm-pore-size mesh), and added to Ritzville and Shano (from Moses Lake, Wash.) silt loams, two soils with low organic matter content (Table 2). Separate experiments were conducted for each soil. Wheat straw was added to the soils at 1.25, 2.5, 5, and 10% (wt/wt) based on soil dry weight. The wheat straw-amended and control soils (no wheat straw added) were sieved (2-mm-pore-size mesh), treated with steam (1 h at 95°C), and air dried on a bench top at room temperature. Three seed treatments (MC, 2-79RN$_{10}$, and 2-79.59.34) were planted into soil infested with *G. graminis* var. *tritici* and evaluated for take-all disease in tube assays as previously described.

Amendment experiments were arranged in a split-plot design with soil amendment as the main plot and seed treatment as the subplot. There were six replicates per treatment with 12 seedlings per replicate for the organic matter tests and five replicates per treatment with 18 seedlings per replicate for the zinc experiment. Disease severity was assessed as described above for the take-all suppression assay. Main effects and interactions were analyzed for significance with the SAS general linear models procedure. Significant effects of qualitative factors were further analyzed with a means comparison test (Fisher's protected least-significant-difference test at P values of ≤ 0.05), while significant effects of quantitative factors were evaluated with single-degree-of-freedom orthogonal polynomials.

RESULTS

Take-all suppression and the relative role of antifungal metabolites. Both main effects (soil and seed treatment) and the interaction of these factors were significant ($P < 0.0001$) for take-all disease rating. When data from all 10 soils were pooled, the PCA-producing strains, 2-79-59.34, 2-79RNL3, and 2-79RN$_{10}$, were more suppressive of take-all than strains that did not produce PCA (Fig. 1). Among the derivatives of 2-79RN$_{10}$ that produced both fluorescent siderophore and anthranilic acid, either of these metabolites singly, or none of the metabolites, there was no difference in disease suppression. However, across all soils, the disease rating was significantly lower for wheat treated with bacterial strains that did not produce PCA than for the control treated with methylcellulose only (Fig. 1).

In 4 of the 10 soils (Larkin, Palouse, Quincy, and Shano #2), all three of the PCA-producing derivatives of 2-79 (2-79-59.34, 2-79RNL3, and 2-79RN$_{10}$) provided significant suppression of take-all compared to the MC control (Fig. 2). At least one of

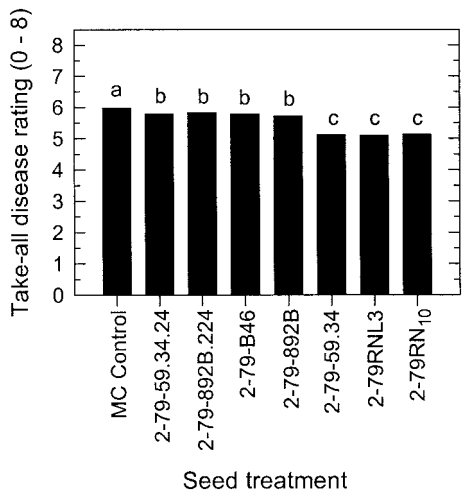

FIG. 1. Effect of seed treatment on take-all disease rating of wheat when data from all 10 soils were pooled. Disease was rated 3 weeks after planting on a scale of 0 to 8 (24). The effect of seed treatment was significant at a P value of ≤ 0.0001. Bars with the same letters are not significantly different according to a Fisher's protected least-significant-difference test at a P value of 0.05.

the PCA producers suppressed take-all in four additional soils (Puget, Shano #1, Walla Walla, and Woodburn). In two soils, Ritzville and Thatuna, the PCA-producing strains failed to protect seedlings against take-all. Strains that did not produce PCA provided some disease control in two soils, Palouse and Walla Walla.

Association of soil factors with biocontrol activity of phenazine-deficient strains. Take-all disease rating of wheat treated with phenazine-deficient, siderophore-producing strains was positively correlated with percent clay and available phosphorous and negatively correlated with copper and available potassium (Table 3). In addition to those four factors, soluble and extractable potassium were negatively correlated with disease rating of seedlings treated with bacterial strains that lacked both phenazine and siderophore (Table 3). There was no significant correlation between soil properties and disease rating of seedlings treated with MC (Table 3).

Soil characteristics associated with biocontrol activity of phenazine-producing strains. Sixteen soil properties were significantly correlated with take-all disease rating of plants treated with 2-79 PCA producers (Table 3). Nine properties, namely, percent total carbon, cation exchange capacity, percent clay, exchangeable acidity, iron, manganese, percent total nitrogen, percent organic matter, and percent silt, were positively correlated with disease rating. As these soil properties increased, biocontrol was diminished and disease increased. Seven soil properties were negatively correlated with take-all disease rating, including ammonium-nitrogen, percent sand, extractable and soluble sodium, soil pH, sulfate-sulfur, and zinc. As these seven factors increased, biocontrol was increased and disease was reduced. In a preliminary PCF analysis, for PCA-producing bacterial treatments only, the take-all disease rating was significantly interrelated with the 16 soil properties. Soil variables not interrelated with disease rating were not included in the final PCF analysis.

Three linear combinations (termed principal components) were responsible for 87% of the covariation among soil properties and disease rating in the final PCF analysis (Table 4). Component 1 had a significant negative loading value for disease rating (−0.71), indicating that disease rating was deemphasized in Component 1. Conversely, component 2 had a significant positive loading (0.63) for disease rating, suggesting that disease rating is emphasized in this component. The loading value for disease rating was not significant (less than the absolute value of 0.35) for component 3 (Table 4).

Within component 1, soil properties with significant positive loadings (ammonium-nitrogen, 0.47; sulfate-sulfur, 0.92; extractable sodium, 0.63; zinc, 0.74; percent sand, 0.84; and soluble sodium, 0.81) were associated with enhanced suppression of take-all by PCA-producing derivatives (Table 4). There were four soil factors with significant negative loadings in component 1 (cation exchange capacity, −0.46; exchangeable acidity, −0.39; percent silt, −0.89; and percent clay, −0.36), suggesting that these factors had a negative impact on biocontrol by PCA producers. Within component 2, where disease rating is emphasized, soil factors with significant negative loadings had a positive impact on biocontrol by PCA producers. These factors included soil pH (−0.91), extractable sodium (−0.55), and percent sand (−0.40) (Table 4). Soil factors with significant positive loadings in component 2, such as organic matter (0.41), cation exchange capacity (0.62), exchangeable acidity (0.73), manganese (0.79), iron (0.86), percent clay (0.74), and total percent carbon (0.40) had an adverse effect on disease control by PCA producers (Table 4).

Regression analysis of soil characteristics associated with biocontrol of take-all by phenazine-producing strains. The 16 soil properties identified with PCF analysis were further analyzed with step-wise least-squares regression. A model was identified ($R^2 = 0.96$; $C_p = 6.17$) that included six key soil properties: ammonium-nitrogen, cation exchange capacity, iron, percent silt, soil pH, and zinc, to explain the variance in take-all disease rating of wheat treated with *P. fluorescens* 2-79RN$_{10}$ and its PCA-producing derivatives.

Interactions among soil properties. In addition to the influence of various soil properties selected by PCF analysis on take-all biocontrol, there were significant correlations among the selected soil properties. Correlations among soil properties are well known. For example, among the six soil properties identified by the regression model, cation exchange capacity was positively correlated with iron and percent silt and negatively correlated with soil pH and zinc (Table 5). Iron was positively correlated with percent silt and negatively correlated with soil pH. Percent silt was negatively correlated with soil pH and zinc, while ammonium-nitrogen was positively correlated with zinc.

Impact of organic matter amendments on disease and biocontrol activity. The effect of organic matter amendment on disease rating of wheat treated with 2-79RN$_{10}$ and 2-79-59.34 was similar in the Ritzville (Fig. 3) and Shano #1 (Fig. 4) silt loams. Biological control with the PCA-producing strains decreased as the percentage of organic matter added to soil increased (Fig. 3A and 4A). At the higher percentages of organic matter (2.5, 5.0, and 10.0%), there was no difference in disease rating between plants treated with 2-79RN$_{10}$ or 2-79-59.34 and the MC control in both soils (Fig. 3B and 4B). For seedlings with no bacterial treatment, the disease rating was

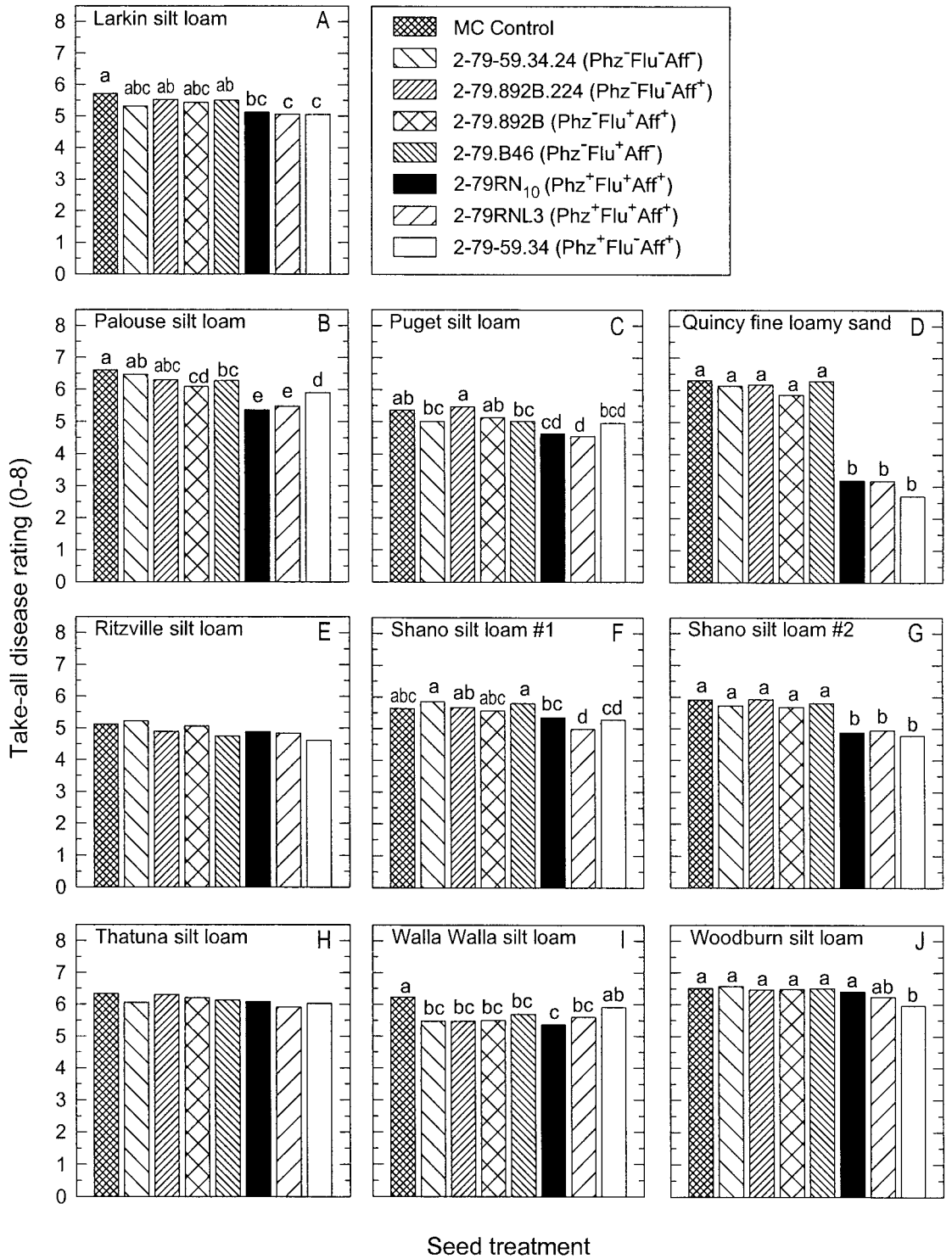

FIG. 2. Effect of the interaction between soil and seed treatment on take-all disease rating. The soil-seed treatment interaction was significant ($P \leq 0.0001$), and further analysis was performed on the response to soil. Within each soil (Larkin silt loam [A], Palouse silt loam [B], Puget silt loam [C], Quincy fine loamy sand [D], Ritzville silt loam [E], Shano silt loam #1 [F], Shano silt loam #2 [G], Thatuna silt loam [H], Walla Walla silt loam [I], and Woodburn silt loam [J]), bars with the same letter are not significantly different according to Fisher's protected least-significant-difference test at $P = 0.05$.

TABLE 3. Correlations between soil properties and disease rating of plants treated with *P. fluorescens* 2-79RN$_{10}$ and derivatives grouped by common phenotype

Soil property	Correlation coefficient for phenotype group (strains)[a,b]:		
	Phz$^+$ (2-79RN$_{10}$, 2-79RNL3, and 2-79-59.34)	Phz$^-$ Flu$^+$ (2-79-892B and 2-79-B46)	Phz$^-$ Flu$^-$ (2-79-892B.224 and 2-79-59.34.24)
Carbon (% total)	0.449**[c]		
CEC[d]	0.765*****		
Clay (%)	0.675*****	0.417*	0.415*
Exchangeable acidity	0.682*****		
Iron	0.624****		
Manganese	0.665*****		
Nitrogen (% total)	0.408**		
Organic matter (%)	0.469***		
Silt (%)	0.736*****		
Ammonium-nitrogen	−0.441**		
Sand (%)	−0.810*****		
Phosphorous (available)		0.507**	0.502**
Copper		−0.497**	−0.414*
Potassium (available)		−0.514**	−0.626***
Potassium (extractable)			−0.406*
Potassium (soluble)			−0.431*
Sodium (extractable)	−0.682*****		
Sodium (soluble)	−0.639*****		
Soil pH	−0.746*****		
Sulfate sulfur	−0.826*****		
Zinc	−0.723*****		

[a] Phz$^+$, produces PCA; Phz$^-$, deficient in production of PCA; Flu$^+$, produces fluorescent siderophore; Flu$^-$, deficient in production of fluorescent siderophore; complete phenotypes of strains are given in Table 1.

[b] No significant correlations were found for seeds treated with MC sticker only.

[c] *, **, ***, ****, *****, correlations were significant at P values of $\leq$ 0.10, 0.05, 0.01, 0.001, and 0.0001, respectively.

[d] CEC, cation exchange capacity.

TABLE 4. Principal component factor analysis (varimax rotation) of disease rating of wheat treated with Phz$^+$ derivatives of *P. fluorescens* 2–79 and soil properties[a]

Soil property	Value for:		
	Component 1	Component 2	Component 3
Disease rating	−0.71	0.63	NS
Soil pH	NS	−0.91	NS
Organic matter (%)	NS	0.41	0.84
Ammonium-nitrogen	0.47	NS	0.82
Sulfate-sulfur	0.92	NS	NS
Sodium (extractable)	0.63	−0.55	NS
Cation exchange capacity	−0.46	0.62	0.43
Exchangeable acidity	−0.39	0.73	0.55
Zinc	0.74	NS	NS
Manganese	NS	0.79	NS
Iron	NS	0.86	NS
Sand (%)	0.84	−0.40	NS
Silt (%)	−0.89	NS	NS
Clay (%)	−0.36	0.74	NS
Sodium (soluble)	0.81	NS	NS
Nitrogen (total %)	NS	NS	0.85
Carbon (total %)	NS	0.40	0.85
Variance	0.64	0.15	0.08
Cumulative variance	0.64	0.79	0.87

[a] Loading values greater than or equal to the absolute value 0.35 indicates significant inter-relatedness within a component; NS, not significant. Phz$^+$ derivatives of *P. fluorescens* 2-79 are 2-79RN$_{10}$, 2-79RNL3, and 2-79-59.34. Phz$^+$, produces PCA.

significantly higher with 10% organic matter amendment in the Ritzville silt loam (Fig. 3A) and with both 5 and 10% organic matter in the Shano silt loam #1 (Fig. 4A).

Impact of zinc amendments on disease and biocontrol activity. Increased concentrations of zinc had no effect on take-all disease severity for MC-treated seeds (Fig. 5A). For plants treated with either 2-79-59.34 or 2-79RN$_{10}$, biological control was enhanced as evidenced by a decrease in disease rating as zinc amendment increased to 50 μg/ml (Fig. 5A). At all concentrations of zinc, disease severity was less with the two PCA-producing strains than with the MC control (Fig. 5B).

DISCUSSION

P. fluorescens 2-79, originally isolated from a take-all suppressive soil in the Pacific Northwest, can protect wheat seedlings against take-all disease when applied to seeds (26, 46, 47). Strain 2-79 produces the antibiotic PCA in the rhizosphere as the primary means of disease suppression (38, 41). The bacterium also produces other antifungal factors and fluorescent siderophore, which play minor roles in disease control compared to PCA (12).

Variability in biocontrol performance of *P. fluorescens* 2-79 in field studies has been reported numerous times (45). Although there are several factors that may impact biological control of root diseases, the influence of the abiotic soil environment is clearly significant. In the present study, under controlled conditions (pathogen density, photoperiod, soil moisture, and temperature), we investigated biocontrol of take-all by PCA-producing 2-79RN$_{10}$ strains in 10 soils. Using interpretational multivariate PCF analysis, we identified soil properties that positively or negatively influenced take-all disease of wheat seedlings in the presence of PCA-producing strains. It is important to note that the soils studied were not deficient in any of the micro- and macroelements evaluated; the soils were steam treated to remove the influence of indigenous microflora and nontarget root pathogens; and soil properties were not significantly correlated with the take-all disease rating of seedlings treated with the MC sticker only (no bacteria).

We identified a negative interrelationship between ammonium-nitrogen, sulfate-sulfur, sodium (extractable and soluble), zinc, percent sand, soil pH, and take-all severity of plants treated with PCA-producing derivatives of 2-79 in the 10 soils. In contrast, cation exchange capacity, exchangeable acidity, percent silt, percent clay, percent organic matter, manganese, iron, and percent total carbon were positively interrelated with take-all severity in the presence of PCA-producing derivatives. In the final PCF analysis, a three-component solution, which included 16 soil properties, accounted for 87% of the variation in disease rating of wheat treated with the PCA-producing derivatives of 2-79. A regression model was developed that included the six soil variables, ammonium-nitrogen, cation exchange capacity, iron, percent silt, soil pH, and zinc, to explain the variance in take-all disease rating of wheat treated with *P. fluorescens* 2-79RN$_{10}$ and its PCA-producing derivatives. Amendment studies with both organic matter and zinc further confirmed their negative and positive influence, respectively, on biocontrol of take-all with PCA-producing 2-79RN$_{10}$ derivatives.

TABLE 5. Correlation coefficients among selected soil properties correlated with disease suppression by Phz^+ strains of *P. fluorescens* 2-79[a]

Soil property	Soil property					
	Ammonium-nitrogen	Cation exchange capacity	Iron	% Silt	Soil pH	Zinc
Ammonium-nitrogen	1.00	0.086 (NS)	0.081 (NS)	−0.284 (NS)	0.042 (NS)	0.377*
Cation exchange capacity		1.00	0.708***	0.571**	−0.680***	−0.580**
Iron			1.00	0.450*	−0.913***	−0.239 (NS)
% Silt				1.00	−0.514**	−0.468**
Soil pH					1.00	0.275 (NS)
Zinc						1.00

[a] Positive and negative correlation coefficients with probability values in parentheses; *, **, and ***, Correlations were significant at P values of ≤0.05, ≤0.001, and ≤0.0001, respectively; Phz^+ derivatives of *P. fluorescens* 2-79 are 2-79RN_{10}, 2-79RNL3, and 2-79-59.34. NS, not significant.

Some of the soil factors that we identified as having a significant influence on biocontrol of take-all by *P. fluorescens* 2-79 have been examined in earlier studies. For example, it is well known that ammonium forms of nitrogen can reduce the severity of take-all (3, 17, 24). Absorption of ammonium-nitrogen by wheat roots leads to excretion of corresponding H^+ ions (33) and a corresponding reduction in rhizosphere pH (36). In slightly acidic to alkaline soils, suppression of take-all has been attributed to the combined effect of direct inhibition of ectotrophic hyphal growth of *G. graminis* var. *tritici* by low pH of the wheat root surface and to specific antagonism by fluorescent pseudomonads in the rhizosphere (34, 35). The decrease in rhizosphere pH associated with ammonium nitrogen also increases the availability of micronutrients, such as manganese (18), to the wheat plant, which is known to influence host susceptibility to take-all (16, 18).

The direct effect of pH on PCA accumulation by 2-79 was tested in liquid fermentation culture by Slininger and Shea-Wilbur (30). Production of PCA was diminished at pHs less than 6, optimal at pH 6 to 7, and declining at pH 7 to 8. In our previous study on the effects of in vitro and in situ pH on inhibition of *G. graminis* var. *tritici* by 2-79RN_{10}, inhibition of

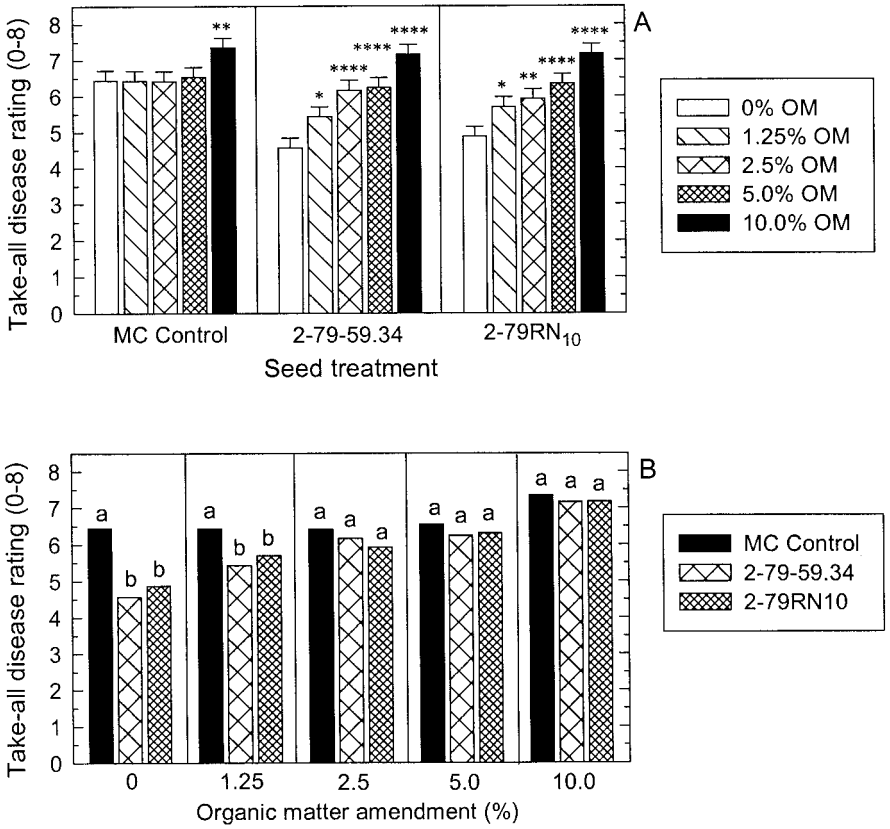

FIG. 3. Influence of organic matter amendment (percentage of wheat straw [wt/wt]) on biocontrol activity of 2-79 in Ritzville silt loam, which is normally low in organic matter (0.93% [Table 2]). (A) Values are the mean ± standard error. Within each seed treatment, each amendment treatment was compared with the 0% control using single-degree-of-freedom orthogonal contrasts. Significant differences are indicated by *, **, ***, and **** at P values of 0.05, 0.01, 0.001, and 0.0001, respectively. (B) Within each rate of organic matter amendment, bars with the same letter are not significantly different according to Fisher's protected least-significant-difference test at a P value of 0.05.

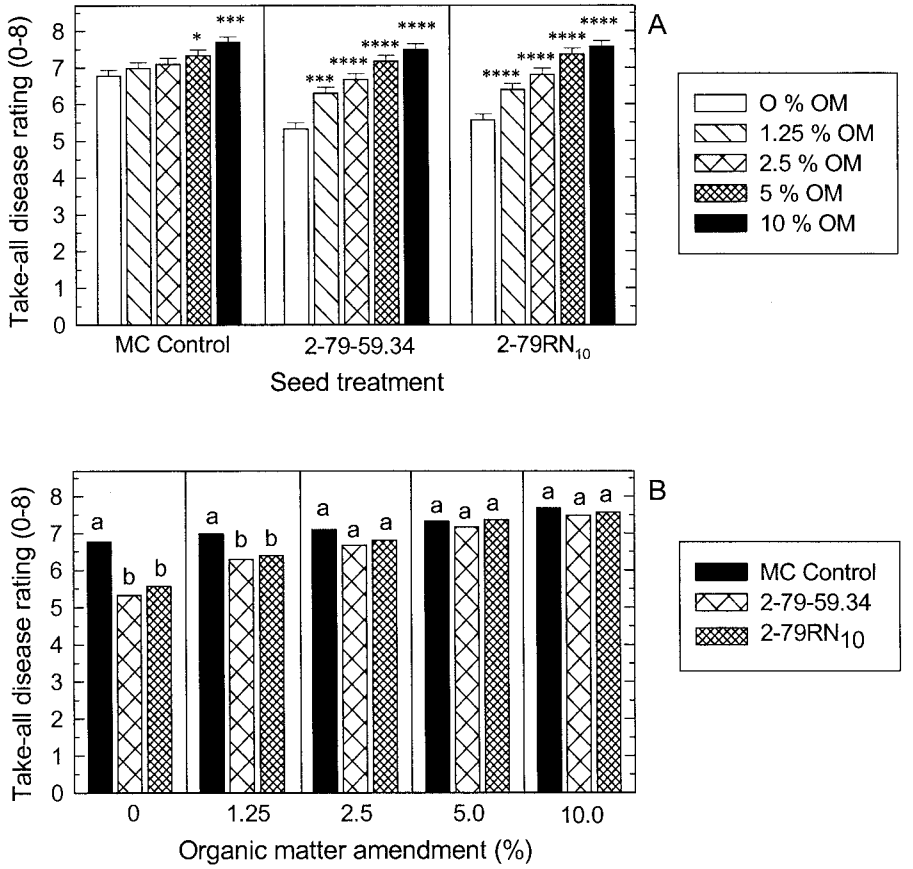

FIG. 4. Influence of organic matter amendment (percentage of wheat straw [wt/wt]) on biocontrol activity of 2-79 in Shano silt loam #1, which is normally low in organic matter (1.13% [Table 2]). (A) Values are the mean ± standard error. Within each seed treatment, each amendment treatment was compared with the 0% control using single-degree-of-freedom orthogonal contrasts. Significant differences are indicated by *, **, ***, and **** at *P* values of 0.05, 0.01, 0.001, and 0.0001, respectively. (B) Within each rate of organic matter amendment, bars with the same letter are not significantly different according to Fisher's protected least-significant-difference test at *P* values of 0.05.

hyphal growth by 2-79RN$_{10}$ in vitro was least at pH 4.9 to 5.8, greatest at pH 6.0 to 6.6, and intermediate at pH 6.8 to 8.0 (26). However, in situ, 2-79RN$_{10}$ significantly reduced take-all in a Ritzville silt loam adjusted to a range of pH values (4.9 to 8.0). In our present study, the pH of the 10 soils ranged from 5.17 to 8.53, and a decrease in pH was associated with an increased disease rating (diminished disease suppression).

In experiments on the effects of minerals on PCA production by *P fluorescens* 2-79 in defined liquid medium, PCA production increased as the concentration of zinc sulfate increased (29, 32). Accumulation of PCA in culture was maximal when a combination of high zinc sulfate (75 μM) and low iron sulfate (36 μM) was added. In the present study, both sulfate-sulfur and zinc were positively associated, while iron was negatively associated, with take-all suppression in soil in the presence of PCA-producing strains. The influence of zinc was further supported by our studies on zinc amendments to a Woodburn silt loam. Increased zinc had no effect on disease of seedlings treated with the MC sticker only, but take-all was significantly reduced in seedlings treated with PCA-producing 2-79RN$_{10}$ strains in soil with the highest rate (50 μg of EDTA-Zn/g of soil) of zinc amendment.

Organic matter content and the amount and type of clay are the main factors that influence cation exchange capacity in soil. Clay and humus provide a large surface area with surface charges to which ions and water are attracted. These surfaces are the centers of activity for chemical reactions and nutrient exchange. In our study, CEC and percentages of clay and organic matter were negatively associated with disease suppression by PCA-producing 2-79 derivatives. To examine effects related to CEC, we manipulated organic matter content in two soils. As suggested by the PCF analysis, increasing the percentage of organic matter led to a corresponding decrease in suppression of disease by 2-79RN$_{10}$ PCA producers in both soils. The bacteria were ineffective in suppressing take-all at higher rates of organic matter amendment (2.5% and above). At the highest rates, 10% in Ritzville silt loam and 5 and 10% in Shano silt loam, failure in biological control was partially due to an increase in disease because take-all was more severe in the "no bacteria" control at high rates of organic matter amendment. The mechanism(s) by which organic matter reduces biocontrol activity of strain 2-79 is a topic of future interest because there is increasing interest in the Pacific Northwest in direct seeding of wheat, which results in accumulation of straw in the field compared to conventionally cultivated fields.

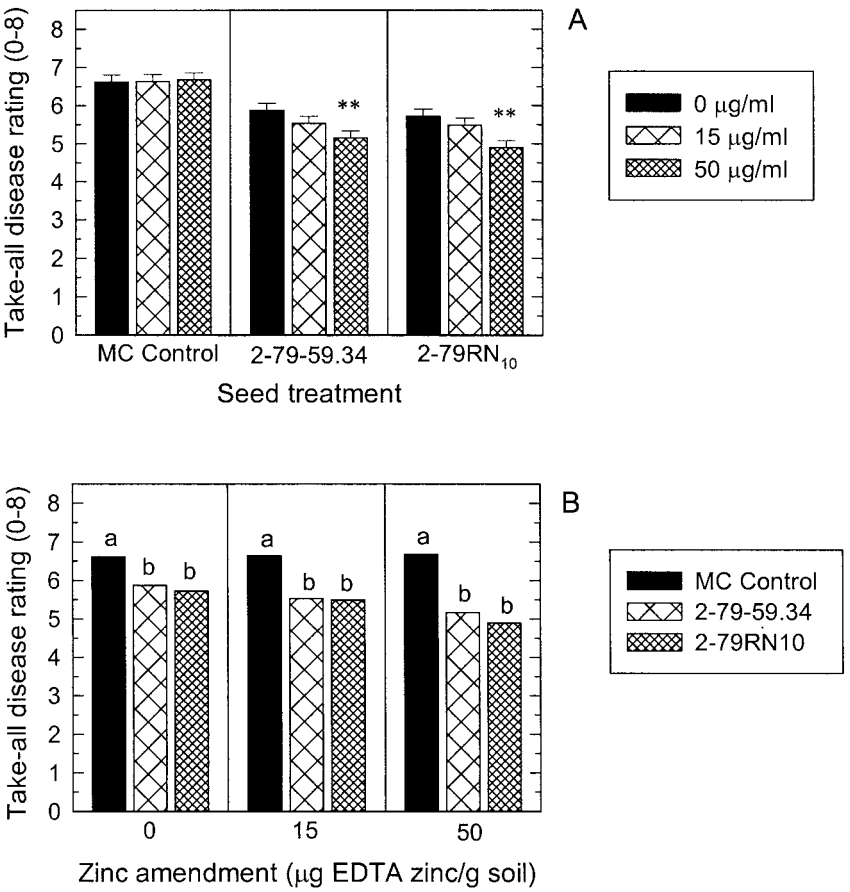

FIG. 5. Influence of zinc amendment (Zn-EDTA, micrograms per gram of soil) on biocontrol activity of 2-79 in a low-zinc soil (Woodburn silt loam [Table 2]). (A) Values are the mean ± standard error. Within each seed treatment, each amendment treatment was compared with the 0% control using single-degree-of-freedom orthogonal contrasts. Significant differences are indicated by double asterisks at a *P* value of 0.01. (B) Within each rate of zinc amendment, bars with the same letter are not significantly different according to Fisher's protected least-significant-difference test at a *P* value of 0.05.

The question remains of how these soil characteristics influence biocontrol activity by PCA-producing 2-79. Factors such as zinc availability and pH could act directly by enhancing production of PCA, while the negative influence of clay, organic matter, and CEC could be related to binding and inactivating PCA, or by adsorbing nutrients needed by the bacteria to effect biocontrol. Potential indirect effects of soil factors on biocontrol of root diseases are numerous. For example, soil factors are known to influence root colonization and survival of bacteria in natural soil (2, 4). Our study also underscores the need for a more complex analysis of the relative role of biocontrol mechanisms. In our study, PCA production was the primary mechanism responsible for take-all suppression by 2-79 in the 10 soils; however, it is possible that in other soil environments, such as those with low iron, mechanisms such as siderophores have a more important role than previously concluded (12). Besides their direct role in sequestering iron and other metals, siderophores may indirectly stimulate biosynthesis of other secondary metabolites involved in biocontrol by increasing the availability of these minerals to the producing bacteria (39). Antibiotics and siderophores may also function to induce local and systemic host resistance (21).

No soil factors were significantly interrelated with take-all severity of nonbacterized seedlings in our study, and there was a sufficiency of macro- and micronutrients in the 10 soils evaluated. However, soil factors can affect susceptibility of the host by altering host plant nutrition (15). Applications of NaCl and other Cl salts to soil infested with *Rhizoctonia solani* increased plant tissue levels of Cl and Mn and the subsequent yield of table beets (11). *G. graminis* var. *tritici* can reduce the availability of Mn in the rhizosphere of wheat through oxidation. Under Mn-deficient conditions, photosynthesis is reduced, metabolism of nitrogen is inefficient, and plant defense reactions against pathogens are limited (16). In the rhizosphere of wheat, biocontrol strains of *Bacillus subtilis* reduced Mn^{4+} to Mn^{2+}, thus making it more available to the wheat plant. Corresponding decreases in take-all severity and increases in yield were reported with these bacteria (16). In contrast, *P. fluorescens* 2-79 can reduce and oxidize manganese (16). Bacteria that can do both may increase disease severity under conditions in which they oxidize Mn in the rhizosphere, thus decreasing availability to the plant. Alternatively, such bacteria may decrease disease if conditions favor Mn reduction, thus increasing its availability to the plant (15). In the present study, Mn was negatively correlated with disease suppression by 2-79;

this may be related to the ability of 2-79 to either oxidize or reduce Mn under different soil conditions.

The interaction between a plant pathogen and a biocontrol agent (8) can be influenced by soil properties. Fusaric acid produced by the phytopathogenic fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici* acts as a repressor of antibiotic production by the biocontrol bacterium *P. fluorescens* CHA0. In hydroponics culturing of tomatoes, addition of Zn reduced fusaric acid production by the pathogen. A concomitant increase in the antibiotic 2,4-diacetylphloroglucinol by CHA0 was reported, and biocontrol was improved (8). The aggressiveness of phytopathogens also can be affected by soil factors. Penetration of wheat roots by *G. graminis* var. *tritici* was greatly reduced when there was a sufficiency of Mn available to plant roots, compared to Mn-deficient conditions (49).

In addition to the many potential impacts of soil factors on a biocontrol system, there is potential for an indirect impact of interactions between factors. For example, soil pH plays an important role in the availability of various macro- and micronutrients. Soil properties that appear to influence disease suppression may not be directly related to biocontrol but may be associated due to strong correlations with other soil factors that are directly related to biocontrol by 2-79.

A practical application of this study and our previous report in which soil factors associated with take-all suppression by the fungal agent *Trichoderma koningii* were identified (10) is that it will provide a basis for customizing biological control treatments against take-all in different soil environments. This could be accomplished by targeting biocontrol agents for sites with favorable soil environments in which they can be expected to perform optimally or by developing formulations with mineral amendments that enhance biocontrol, as shown with zinc and 2-79. Through studies with liquid fermentation culture of 2-79, Slininger and colleagues (31) have developed formulations encapsulated on wheat seed that have improved viability, reduced phytotoxicity, and enhanced take-all suppression. Identification of soil factors that influence biological control will provide a biological basis for improved integration of biological controls with cultural practices that manipulate soil properties, with an aim toward improved disease control. The influence of soil factors on biological control is also an argument in support of incorporating a variety of biocontrol mechanisms in inoculants, perhaps through combinations of compatible organisms (27).

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